Engineering microbes to sense and eradicate *Pseudomonas aeruginosa*, a human pathogen

SUPPLEMENTARY INFORMATION

Supplementary Figure 1-2, Supplementary Table 1

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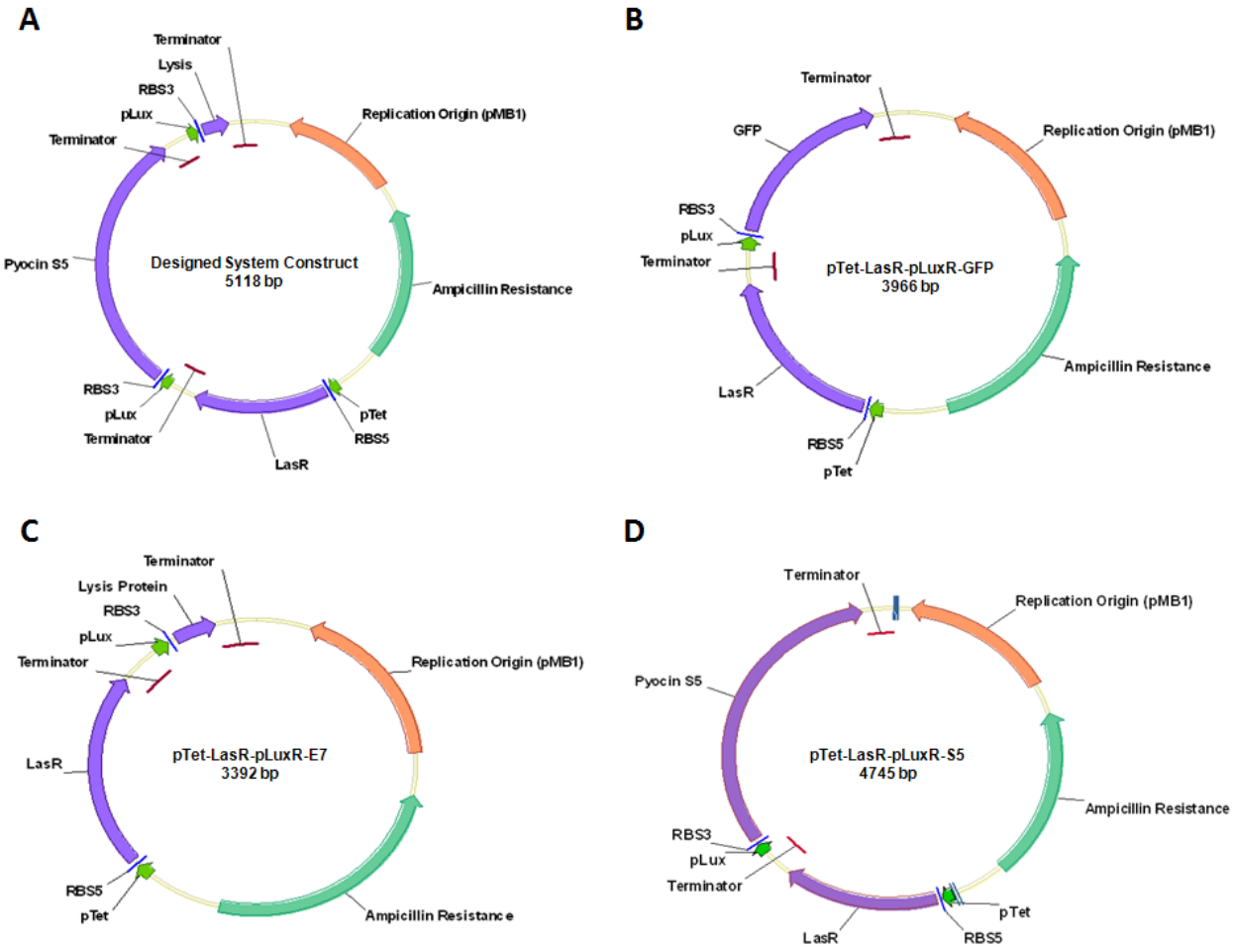
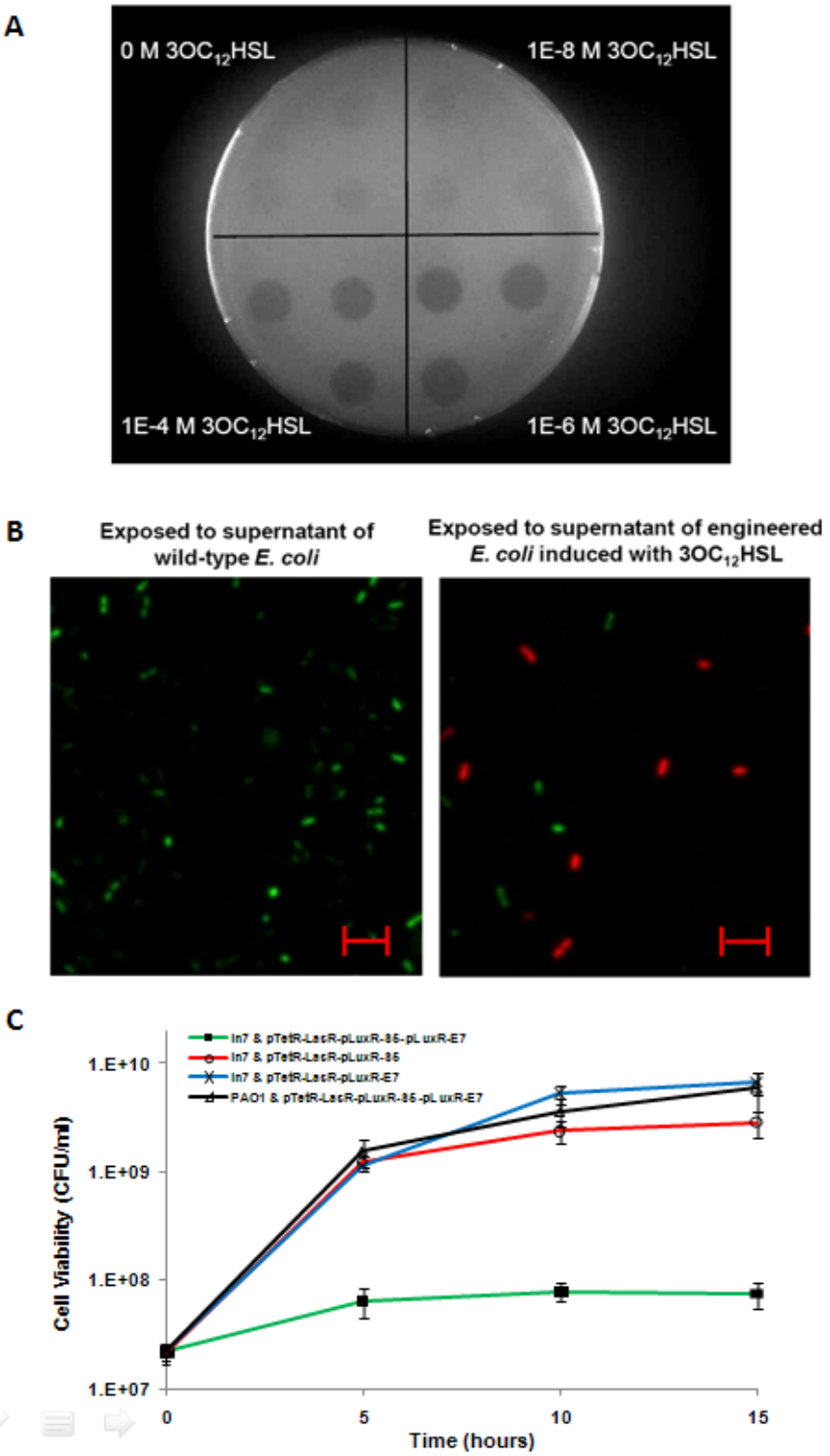


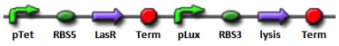
Supplementary Figure 1 Genetic maps of engineered system/devices in pSB1A2 vector. (**A**) Our final engineered system, pTetR-LasR-pLuxR-S5-pLuxR-E7. The system recognizes input chemical signals from *P. aeruginosa* and produces S5 pyocin and E7 lysis proteins. (**B**) Sensing device coupled to GFP, pTetR-LasR-pLuxR-GFP. This construct was used as a measurement tool for characterization of the sensor (pTetR-LasR-pLuxR-). (**C**) Sensing device with E7 lysis**,** pTetR-LasR-pLuxR-E7. This construct was used for the characterization of E7 lysis protein whose function is to disrupt cell membrane for the release of pyocin. (**D**) Sensing device with pyocin S5, pTetR-LasR-pLuxR-S5. This construct was used as a control to compare the efficiency of lysis device in mediating protein release.



Supplementary Figure 2 Inhibition of *P. aeruginosa* by the engineered *E. coli* induced with $3OC_{12}HSL$. (**A**) Agar overlay assay of *P. aeruginosa* after exposure to supernatant of the *E. coli* carrying pTetR-LasR-pLuxR-S5-pLuxR-E7 (the final system) at different $3OC_{12}HSL$ concentrations. Faint inhibition areas were observed with 0 M and 1.0E-8 M $3OC_{12}HSL$. The results show that supernatant of engineered *E. coli* culture induced by 1.0E-6 M $3OC_{12}HSL$ produced wider and clearer inhibition zones relative to other inducer concentrations. (**B**) *P. aeruginosa* cells stained using the

LIVE/DEAD cell viability assay. The results show that more PI-stained *P. aeruginosa* cells were present when *P. aeruginosa* was exposed to supernatant of the *E. coli* carrying the final system that was induced by $3OC_{12}HSL$, whereas all *P. aeruginosa* cells exposed to supernatant of wild-type *E. coli* (control) were stained with SYTO 9 (green). Scale bar: 5 μm. **(C)** CFU count of *P. aeruginosa* (carrying chloramphenicol-resistant plasmid pAWG1-1) in a mixed culture with engineered *E. coli*. To study whether the engineered *E. coli* carrying the final system can inhibit growth of *P. aeruginosa* in mixed culture, clinical isolate ln7 and pyocin resistant control strain PAO1 was co-cultured with engineered E. coli in the ratio 1:4 and quantified by viable cell count of *Pseudomonas*. Additionally, ln7 was also co-cultured with control *E. coli* missing either the pyocin S5 or E7 lysis devices. The results show that only the final system (i.e. pTetR-LasR-pLuxR-S5-pLuxR-E7), complete with sensing, killing and lysis devices are capable of inhibiting the growth of *P. aeruginosa* for 15 hours. Error bar represents the standard deviation of 3 independent replicates.

Supplementary Table 1 List of all genetically encoded parts, devices and systems used in this work. The part number, functional description and symbol used are listed for each component. Descriptions of all BBa parts can be found in the Registry of Standard Biological Parts while the rest are explained in text.

Part Number	Description	Symbol
BBa_R0040	TetR repressible promoter	
BBa_R0062	LuxR & HSL inducible promoter	
BBa_B0032	Ribosome Binding Site (medium)	
BBa_B0034	Ribosome Binding Site (strong)	
BBa_C0179	LasR coding region	
BBa_E0040	Green Fluorescence Protein	
BBa_K117000	Lysis Protein	
BBa_B0015	Terminator	
pTetR-LasR-pLuxR-	Sensing Device	
pTetR-LasR-pLuxR-GFP	Sensing Device with Reporter protein	
pTetR-LasR-pLuxR-S5	Sensing Device with Pyocin S5	
pTetR-LasR-pLuxR-E7	Sensing Device with ColicinE7 lysis gene	
pTetR-LasR-pLuxR-S5-pLuxR-E7	Sensing/killing system construct	